Thin-Layer Chromatographic Separations of Seed Oil Unsaponifiables from Four *Sesamum* Species

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A new, two-dimensional thin-layer chromatographic system was established to provide good separation of the unsaponifiable fractions from the seed oils of three wild *Sesamum* species, [*S. alatum*, Thonn.; *S. radiatum*, Schum and Thonn.; and *S. angustifolium*, (Oliv.) Engl.] and of the cultivated *S. indicum*, L. The system utilizes silica gel plates and n-hexane/diethyl ether (7:3, v/v) and chloroform/diethyl ether (9:1, v/v) as mobile phases in the first and second directions, respectively. The system could be used for qualitative studies and as a preparative technique for subsequent quantitative gas chromatographic separations in chemotaxonomic and related studies on *Sesamum* spp.

KEY WORDS: Analytical and preparative TLC, sesame seed oil, sesamin, sesamol, sesamolin, sterols, two-dimensional TLC, unsaponifiables.

Oil from cultivated sesame seed (*Sesamum indicum*, Linn.) is markedly different from all other vegetable oils in many chemical, biological and physiological properties. Most of these unusual characteristics are due to its content of the unique unsaponifiable constituents, sesamol, sesamin and sesamolin (1).

Wild species of the genus *Sesamum* contain considerable amounts of seed oil. Since these oils have not been investigated for their lipid composition, we studied three wild species growing in Sudan. These are *S. alatum*, Thonn.; *S. radiatum*, Schum and Thonn.; and *S. angustifolium*, (Oliv.) Engl. In a previous paper (2), we reported fatty acid and triacylglycerol compositions of the above three species as compared to those of different cultivars of cultivated *S. indicum*.

Sesamin and sesamolin together with sterols, constitute the major fraction of the unsaponifiable matter in *S. indicum* oil. Bedigian *et al.* (3) screened seed oils from eleven wild *Sesamum* species for the presence of sesamin and sesamolin during their studies on the origin of sesame. They reported that *S. angustifolium* contained both sesamin and sesamolin, *S. radiatum* contained only sesamin and *S. alatum* was void of both lignans.

There are three sterol classes—the desmethyl, monomethyl and dimethyl sterols (4). These are normally analyzed by gas chromatography (GC) after preparative separations on silica gel plates. Itoh *et al.* (5,6) analyzed the sterols of sesame oil after thin-layer chromatography (TLC) separation of the unsaponifiable material on silica gel plates with n-hexane/diethyl ether (HDE; 8:2, v/v) as mobile phase. With that system it was not possible to provide pure fractions for the three sterol classes because sesamin and sesamolin co-occurred with the mono- and dimethyl sterols, respectively. Gaydou *et al.* (7) used a chloroform/diethyl ether (CDE; 9:1, v/v) phase to fractionate the unsaponifiables of some other seed oils. During the course of our studies on the sterols from the four *Sesamum* species, this solvent provided a better separation (compared to the HDE) for the unsaponifiables of *S. indicum*, *S. radiatum* and *S. angustifolium*, but not for those of *S. alatum*. To achieve appropriate separations for the unsaponifiable fractions from all of the four oils that can be used as a preparative technique for subsequent GC analyses, a two-dimensional TLC system was developed by combining the solvent systems used by Itoh *et al.* (5) and by Gaydou *et al.* (7). In this paper, we describe the system, as well as its applications in the analysis of the unsaponifiable fractions of oils from wild *Sesamum* species.

MATERIALS AND METHODS

Seeds from *S. indicum*, L. (variety Kenana 1) were obtained from Kenana Research Station, Agricultural Research Corporation (ARC), Sudan. Seeds of the three wild species [*S. radiatum*, Schum and Thonn.; *S. angustifolium* (Oliv.) Engl.; and *S. alatum*, Thonn.] were collected from different locations in the Sudan (2). All solvents used were of analytical grade (Merck, Darmstadt, Germany) and were used without further purification. TLC plates, pre-coated silica-gel 60 plates, 20 × 20 cm, 0.25 mm layer thickness, were obtained from Merck. The reference sterols and sesamol were obtained from Sigma Chemical Comp. (St. Louis, MO). Purified sesamol, sesamin and sesamolin standards were a gift from Dr. Yasuko Fukuda (Ichimura Gakuen Junior College, Inuyama, Aichi, Japan).

Extraction and saponification. Oven-dried sesame seeds (103°C, 4 hr) were extracted in a Soxhlet apparatus with n-hexane. Saponification was achieved by refluxing the oils (*ca.* 5 g) for 1 hr with 50 mL of 1M ethanolic potassium hydroxide (8). The unsaponifiables were extracted twice with diethyl ether (2 × 40 mL).

One-dimensional TLC. Portions of the unsaponifiables (*ca.* 1.5 mg) were applied as 1.5-cm bands on TLC plates with an autoapplicator, Linomat 3 (CAMAG, Muttenz, Germany). Sesamol, sesamin, sesamolin, γ-tocopherol, cholesterol and cycloartenol were applied as reference spots on both sides of the plate. TLC separations were performed with CDE as mobile phase. The plates were sprayed with 50% H_2SO_4 and heated at 110°C for 5–10 min for qualitative analysis.

Two-dimensional TLC. Two-dimensional TLC was performed on the same type of plates with HDE as developing solvent in the first direction and CDE in the second direction. Plates were also sprayed with 50% H_2SO_4 and heated at 110°C for 5–10 min for qualitative analysis, and other plates were exposed to iodine vapor for a few seconds (when required) as a preparative technique for further quantitative measurements.

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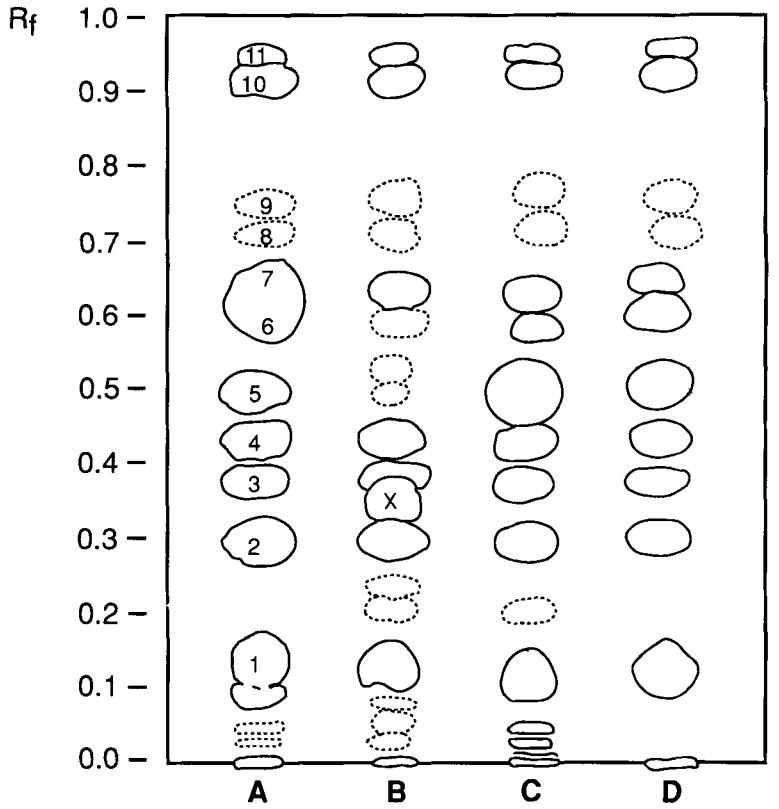

FIG. 1. TLC separation of the unsaponifiables of (A) *Sesamum indicum*, (B) *S. alatum*, (C) *S. radiatum*, and (D) *S. angustifolium* on silica-gel 60 plates with chloroform/diethyl ether (CDE; 9:1, v/v) as mobile phase. Spots: (2) Desmethylsterols, (3) monomethylsterols, (4) dimethylsterols, (5) sesamin, (6) sesamolin, (7) tocopherols, and (1,8–11) unknowns.

RESULTS AND DISCUSSION

Oil from *S. indicum* (variety Kenana 1) contained 1.2% unsaponifiables. Oil from the wild species had higher unsaponifiable contents: *S. radiatum*, 2.3%; *S. angustifolium*, 3.3%; and *S. alatum*, 4.2% (unpublished results).

The one-dimensional thin-layer chromatogram for the separation with CDE of the unsaponifiables of the three wild *Sesamum* species as compared to those of the cultivated *S. indicum*, L. is given in Figure 1. Eleven major spots were observed, with intensities differing from one species to another. Spots 2–4 were identified as desmethyl- (Rf 0.30), 4-monomethyl- (Rf 0.37) and 4,4-dimethylsterols (Rf 0.43), respectively. Sesamol occurred with the monomethyl sterols. Sesamin and sesamolin were identified as spots 5 and 6 at the Rf values of 0.50 and 0.60, respectively. γ-Tocopherol occurred as spot 7 at 0.63. The first diffuse spot at Rf 0.15, the two spots (8 and 9) at Rf values of 0.72 and 0.75, and the two spots (10 and 11) at Rf values of 0.93 and 0.95 were not identified. Some other minor spots were also observed.

Chromatograms of two-dimensional TLC are shown in Figure 2. The composition of *Sesamum* oil unsaponifiables seemed complicated and two-dimensional TLC provided better fractionation and showed several variations between the cultivated and wild species, both in the number of spots and their relative intensities. This two-dimensional system was utilized to obtain pure sterol fractions (unpublished results).

S. alatum had a different TLC pattern with a predominant unknown spot (X) extending from the monomethyl- to the dimethyl sterol areas in the one-dimensional system. This spot represented about 1% of the oil from this species. It dominated the mono- and dimethylsterol fractions and limited the utility of the CDE solvent in obtaining pure fractions for these sterols. In the two-dimensional system, *S. alatum* also yielded the most complex pattern, with many spots at the base line of the HDE solvent and only minor spots for sesamin and sesamolin. The large spot (X) is centered at Rf 0.35 (CDE) in the two-dimensional system. The compound responsible for this spot was isolated and is now under investigation. This compound was a furofuran lignan related to sesamin (unpublished results). *S. angustifolium* also showed two spots with Rf = 0 in the HDE system. The identity of the

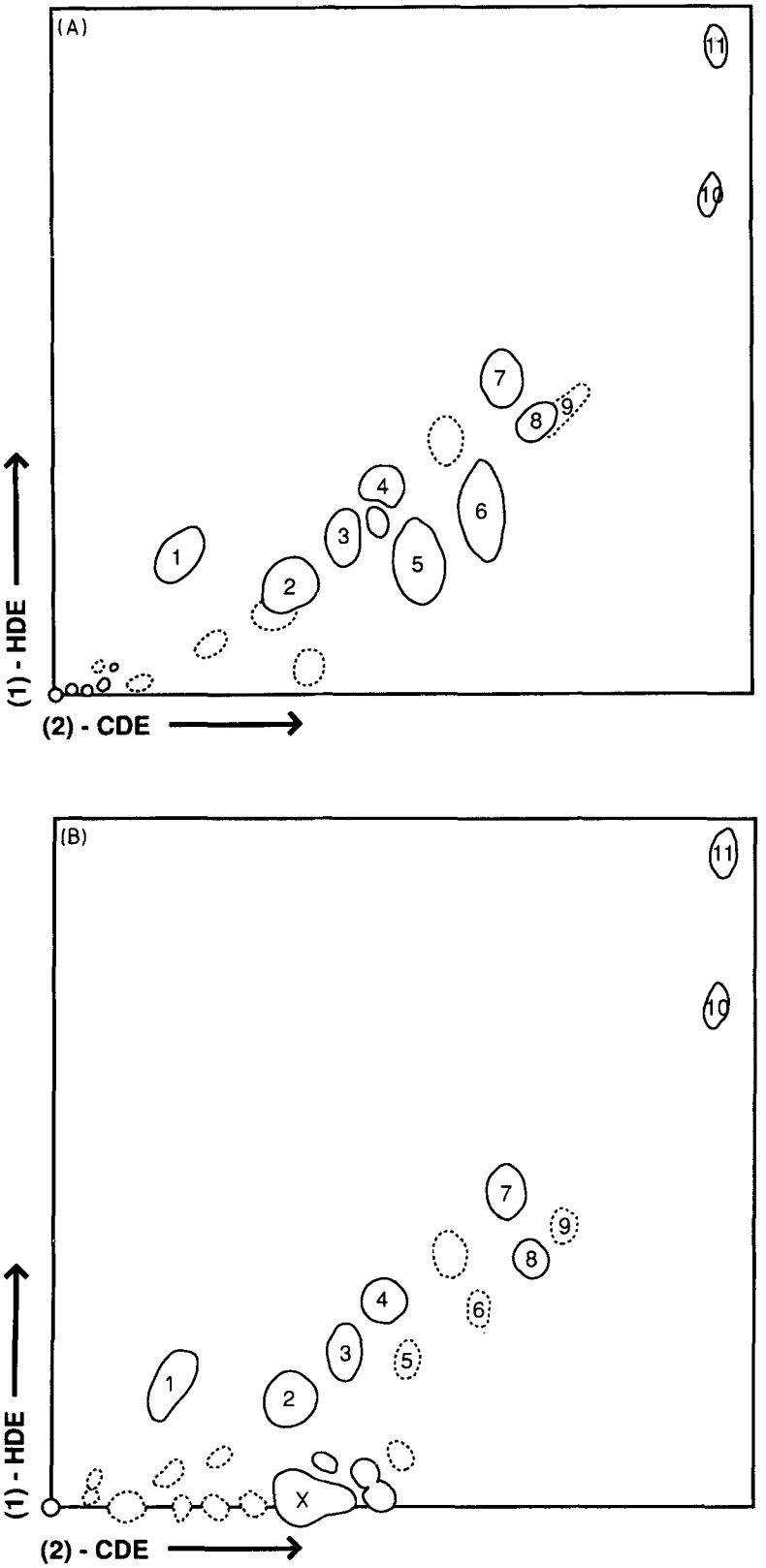

FIG. 2. Two-dimensional TLC separation of the unsaponifiables of (A) *Sesamun indicum*, and (B) *S. alatum* on silica-gel 60 plates, with the solvents (1) HDE, n-hexane/diethyl ether (7:3, v/v); and (2) CDE, chloroform/diethyl ether (9:1, v/v). Spots: (2) Desmethylsterols, (3) monomethylsterols, (4) dimethylsterols, (5) sesamin, (6) sesamolin, (7) tocopherols, and (1, 8–11) unknowns.

nonsterols observed in this fractionation is presently under study and will be reported in subsequent papers.

This two-dimensional system can be used to provide pure fractions in preparative experiments for further GC qualitative and quantitative measurements of the seed oils of other wild *Sesamum* species, and probably of other related genera. The improved resolution in this system as compared to one-dimensional systems (3,7) will be valuable in qualitative screening studies on the identity and relative amounts of the sesamin-type compounds in *Sesamum* oils.

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